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Plant physiologist amongst the grain legumes

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Abstract

This paper expresses a physiological approach to plant functioning based on studies of the efficiency of operation of plant organs and whole plants. Applied to nodulated legumes, it has the purpose of discovering how efficiently photosynthetic resources are utilized for nitrogen fixation and the synthesis of protein in vegetative parts and seeds. Comparisons between species suggest how differences in structure of organs and in growth habit and reproductive behaviour of the legume may influence its efficiency of functioning and yield of seed. The techniques illustrated for studying the economy of carbon and nitrogen might well be applied widely in studies of the productivity of non-leguminous crops.

Introduction

According to figures published by the Food and Agricultural Organization of the United Nations (FAO) the annual world yield of cereals and grain legumes amounts to approximately 1 500 million tonnes of harvested seed, the equivalent of three-quarters of the food dry matter used for human consumption. Although production of cereals within this total is more than ten times greater than that of legumes, the high protein content of legume seeds (Table 1), and the high levels of certain essential amino acids in their seed protein make legume grain uniquely important in the nutrition of man and his animals, especially when complementing a predominantly cereal diet (Siegel and Fawcett 1976).

Despite their importance to man most species of grain legumes have been subjected to much less extensive research than have cereals and other major crop plants. This becomes apparent when examining the volume of literature generated annually on different crops, the extent of national and international funding for research on leguminous versus non-leguminous crops, and the deployment of scientific personnel within research institutes concerned with the breeding and physiology of agriculturally-important plants.

The sad state of grain legumes on the world scene is also obvious when one follows changes in their productivity over the past twenty years, as indicated by yield figures given by the FAO. During this period average yields of cereal crops per hectare have risen by some 65%, due especially to the development of high-yielding cultivars of rice, wheat and maize, but little or no improvement is evident over the same period in productivity of most of the major grain legumes. Even in those species subjected

to intensive selection programmes, for example soybean (*Glycine max* (L) Merr.) and garden pea (*Pisum sativum* L.) the improvement in yield per hectare over the 20-year period has been modest—of the order of 20-35%. This situation, combined with the low baseline productivity of legume crops as a whole (Table 1), the uncertainty of yield realization from one season or soil type to the next, and the notorious susceptibility of grain legumes to diseases, pests and environmental stress, would make one doubt whether further increases in funds and manpower should be devoted to their exploitation as crop plants.

Despite this distressing situation much can still be marshalled in defence of grain legumes. Firstly, it may be argued that their usefulness and productiveness should be assessed primarily in terms of protein yield. When viewed on this basis the comparisons with cereal crops become much more favourable, with several legume species outyielding cereals in protein per hectare by a considerable margin (Table 1). Moreover, in the case of peanut (*Arachis hypogaea* L.) and soybean a second valuable product, oil, is present in large amount in the seed. Secondly, the role of legumes in crop rotations should be recognized. In many parts of Asia, for example, grain legume crops are often grown at times of the year not suitable for cereals and under climatic conditions inferior to those allotted to the cereal. Lower yields from the legume are to be expected under these conditions, but, the fact that a second, nutritionally-valuable crop can be included in the year's cultivation, commends use of the legume. Thirdly, many grain legumes are short-cycle crops, occupying the ground for a much shorter period of time than in the case of many cereal crops; again comparisons based on yield of seed per hectare are unfair. Finally,

Table 1

World production and average yields of major grain legumes and cereals

Grain legumes	Pro- duction 1974 (million tonnes/yr)	Average yield 1965-74 kg/ha	Protein level in dry weight of seed %	Average seed protein yield 1965-74 kg/ha	Major producing countries (in order of importance as producers) 1974*
Soybean (<i>Glycine max</i> (L.) Merr.)	56.8	1 295	38.5	499	USA, China, Brazil, Indonesia, Mexico, USSR, Canada
Peanut (in shell) (<i>Arachis hypogaea</i> L.)	17.6	879	28.6	251	India, China, Nigeria, Senegal, Sudan, Burma, USA
Dry beans (<i>Phaseolus</i> spp.)	11.5	484	26.0	126	Brazil, India, China, Africa, USA, Mexico, Europe
Dry pea (<i>Pisum sativum</i> L.)	11.7	1 117	23.0	257	USSR,† China, W. Europe, USA, Ethiopia, S. America
Chickpea (<i>Cicer arietinum</i> L.)	5.9	617	19.9	123	India, Pakistan, Mexico, Ethiopia, Turkey, Burma
Broad bean (<i>Vicia faba</i> L.)	5.2	1 076	27.5	296	China, Italy, Egypt, U.K., Morocco, Ethiopia, Brazil
Vetches (<i>Vicia</i> spp. <i>Lathyrus</i> spp.)	2.1	1 241	27.5	341	USSR, Turkey, Spain, Poland, Morocco, Greece
Pigeon pea (<i>Cajanus cajan</i> (L.) Millsp.)	1.5	653	22.1	144	India, Africa, Latin America
Cowpea (<i>Vigna unguiculata</i> (L.) Walp.)	1.1	330	26.4	87	Nigeria, Upper Volta, Uganda, Asia, USA
Lentil (<i>Lens esculenta</i> Moench.)	1.2	609	24.5	149	India, Turkey, Ethiopia, Syria, USSR, W. Europe
Lupin (<i>Lupinus</i> spp.)	0.8	828	34.5	286	USSR, Poland, Australia, W. Europe
Others	3.3	522	
Total legumes	118.7	
Total cereals‡	1.357	1 750	11	193	

* Calculated from average values for seed protein content given in the Table.

† Where the name of a country is printed in italics its contribution accounts for more than half of the world's production.

‡ Includes wheat, barley, maize, sorghum and rice.

the unique contribution made by legume crops to soil fertility through their nitrogen-fixing symbiosis with *Rhizobium*, must be recognized. According to data provided by Nutman (1976) this can amount to 60-210 kg N per hectare per growing season. The attendant savings in reduced input of synthetic nitrogen fertilizers are likely to be quite substantial, whether in terms of expense to the farmer, or, indirectly, as a reduction in run-off of nitrogenous fertilizer residues into surrounding ecosystems.

The challenge then to students of the grain legumes is to assess from a variety of viewpoints the future value of these plants on the world agricultural scene. Agronomists will contribute by indicating how better management practices can lead to higher seed yield under a range of soil types and environments. Plant breeders will contribute by developing high-yielding cultivars resistant to disease, adapted to specific environments, and productive of better quality protein. The research of plant physiologists is likely to provide a fundamental understanding of how legumes function and an indication of the processes likely to limit production of seed protein under a range of agricultural conditions.

Questions relating to the productivity of plants require detailed knowledge of the integrated functioning of the whole plant and its parts and investigations of the vast spectrum of related events and processes at structural, physiological and biochemical levels which accompany development of the plant and its organs during growth. The purpose of this paper is to highlight this type of approach as applied to grain legumes, concentrating particularly on studies conducted by the author and his colleagues in Belfast, Northern Ireland and currently at the University of Western Australia. The first section of the paper deals with investigations of the functioning of nodulated roots, especially in relation to their utilization of carbohydrate for nitrogen fixation. The second section concerns the developing legume fruit and the mechanisms involved in its conversion of imported assimilates into the reserves of seeds. The third section is an examination of the functioning of the whole grain legume, in an attempt to visualize how it deploys its photosynthetic product for vegetative growth, nitrogen-fixation, and the storage of protein in seeds.

The functioning of nodulated roots

The primary function of plant roots is to supply minerals, water, and certain synthetic products to the shoot system. In legumes the synthetic functions of below-ground parts assume unusual importance, due to the nitrogen-fixing activities of nodules or the assimilation in roots of combined forms of nitrogen such as ammonium or nitrate (Pate and Wallace 1964; Wallace and Pate 1965; Oghoghorie and Pate 1971).

It is now generally agreed that ammonia is the first stable product formed during nitrogen fixation by the bacteroids of the nodule (Bergersen 1971; Dilworth 1974), and that its assimilation into organic compounds probably takes place via the glutamate synthase/glutamine synthetase system of the host cytoplasm of the bacterial tissue (Miflin and Lea 1977). From the glutamine produced by this system, other secondary products form, these and glutamine being exported from the nodule to the plant shoot through the transpiration stream (Pate 1971).

Decapitated roots and detached nodules of grain legumes commonly bleed a fluid from their xylem, and this can be readily collected and analysed to reveal which compounds are being utilized for export of fixed nitrogen. Some grain legumes (e.g. *Lupinus* spp., *Pisum* spp. and *Vicia* spp.) export upwards of 70% of their fixed nitrogen as the amides glutamine and asparagine, the latter compound usually predominating (Pate *et al.* 1969; Gunning *et al.* 1974), whilst in other legumes (e.g. *Phaseolus* spp., *Vigna unguiculata* (L.) Walp.) the ureides allantoin and allantoic acid are the principal exports (Atkins *et al.* 1978). These various nitrogenous products are readily labelled with ^{14}C and ^{15}N following feeding of the legume shoot with $^{14}\text{CO}_2$ and its root gas space with $^{15}\text{N}_2$ (Pate 1962; Oghoghorie and Pate 1972; Herridge *et al.* 1978), indicating that the compounds in question are true metabolic products of the fixation process.

Roots are of course dependent on carbohydrates received from the shoot as translocated sugar. Some of this carbohydrate is consumed in respiration of the nodulated root, some is used in formation of dry matter during growth of roots and nodules, and some serves as carbon source for formation of the fixation products which ultimately leave the root in the transpiration stream. The extent of these different forms of usage of carbohydrate has been assessed during growth of two legumes, white lupin (*Lupinus albus* L.) (Pate and Herridge 1978) and cowpea (*Vigna unguiculata*) (Herridge and Pate 1977). Carbon incorporated during growth is assessed by measurements of dry weight gains of root and nodules over specific intervals of growth. The respiratory output of the enclosed nodulated root of the intact plant is measured continuously by a gas-flow system involving collection of respired CO_2 in Pettenkoffer assemblies (Minchin and Pate 1973), and the proportion of this CO_2 efflux due to nodules is estimated by comparing the specific activity of respiration

of freshly detached nodules with that of their supporting root. Finally, the types and concentrations of compounds in xylem exudate of the nodulated root are assessed, and, by relating the C:N ratio of these exports to the total nitrogen increment of the shoot, the amount of carbon cycled through the root as fixation products is estimated.

In both cowpea and white lupin the carbon used in export of fixed nitrogen comprises the largest item of the carbon budget of the nodule, respiratory losses from the nodule the next largest item, and the incorporation of carbon into nodule dry matter the least expensive item. The carbon balance of the whole nodulated roots shows root respiration to be the largest consumer of carbohydrate. The large tap root of lupin is clearly more expensive to maintain than the less extensive root system of the cowpea. When their consumption is compared in relation to how much nitrogen they fix, nodules of lupin are found to use carbon less efficiently than those of cowpea (Table 2; Fig. 1). One reason for this is the higher C:N ratio of the amides exported from lupin nodules than of the ureides exported from cowpea nodules, but CO_2 efflux per unit of nitrogen fixed is also greater from nodules of lupin than of cowpea (Table 2), suggesting that other elements are involved in efficiency of operation.

Table 2.

Economy of carbon in nitrogen-fixing nodules of two grain legumes.*

Species	<i>Vigna unguiculata</i>	<i>Lupinus albus</i>
Period of growth	0.78†	0.94†
	days	
	mg.plant ⁻¹	
N fixed	726	788
N exported	705	761
C used in export of N. ‡	969	1789
C lost as CO_2 in nodule respiration	789	1372
C incorporated into nodule mass	253	298
Total C requirement of nodule	2 011	3 459
	g atom . g atom ⁻¹	
Total C required/N fixed	3.23	5.12
C exported/N exported	1.60	2.74
C respired/N fixed	1.27	2.02

* Data from Herridge and Pate 1977; Pate and Herridge 1977; Atkins, Herridge and Pate 1978.

† Vegetative growth and early fruiting.

‡ Estimated from the amount of N exported and the C:N ratio of xylem sap.

Since nodules have a relatively high rate of consumption of translocate in proportion to their mass, their functioning in nitrogen fixation is especially vulnerable to a decline or interruption in supply of photosynthate from the shoot. How-

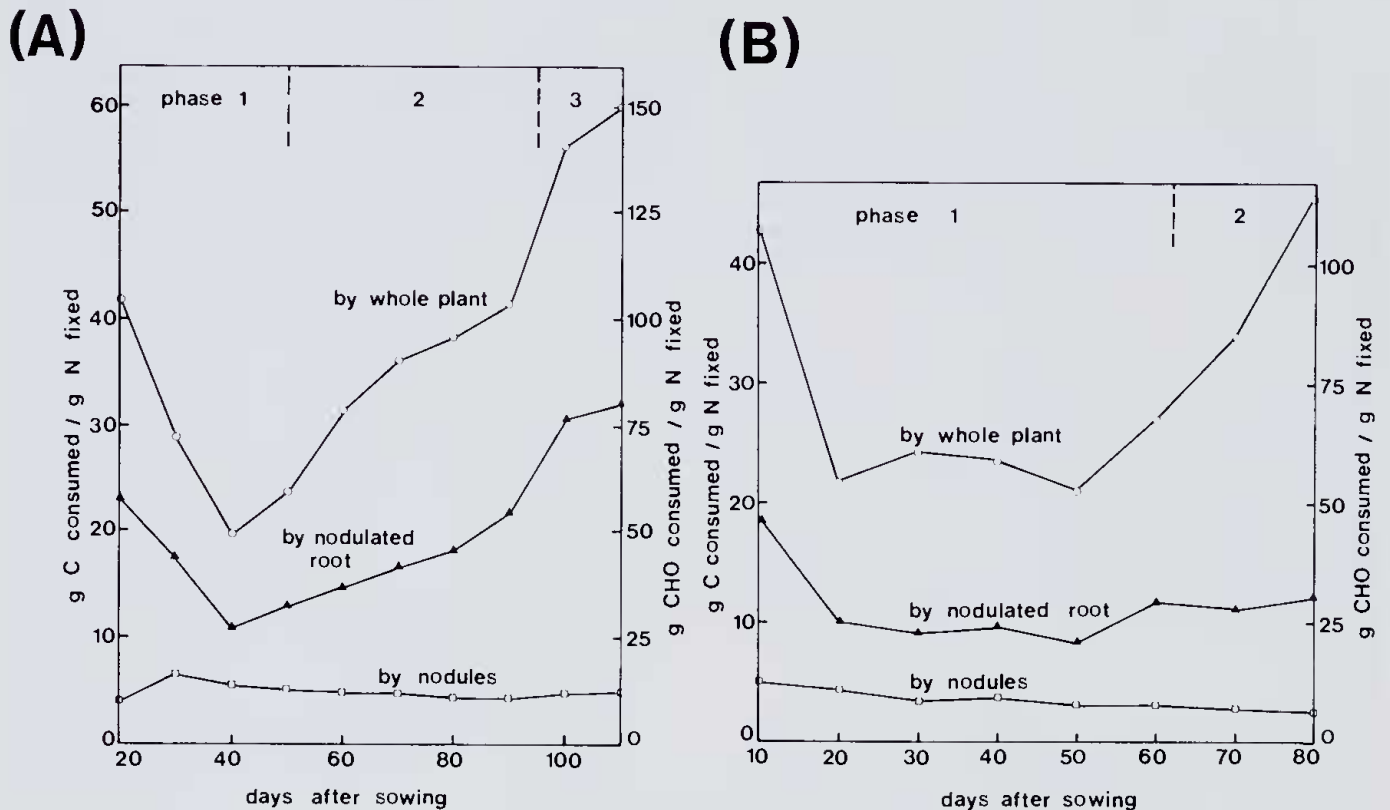


Figure 1.—The cost in terms of carbon consumption (as net photosynthate) of nitrogen fixation by nodules, nodulated root or whole plant over the life cycles of two grain legumes. (A) *Lupinus albus* L. (cv Neutra), and (B) *Vigna unguiculata* (L.) Walp. (cv Caloona). Phase 1 refers to vegetative growth before flowering, Phase 2 to flowering and early fruiting, Phase 3 to the final stages of fruiting. Data of (A) from Pate and Herridge 1978, and of (B) from Herridge and Pate 1977.

ever, they appear to possess a strategy of diurnal functioning in which sugar and polysaccharide built up during the day serve as carbon sources for fixation at night, thus allowing the nodule to fix much more nitrogen at night than would be possible were it to have to rely solely on current translocate (Minchin and Pate 1974). In garden pea (*Pisum sativum* L.) and cowpea, removal of the shoot causes within 24-48 hours a marked decline in the nitrogen-fixing activity of the root, as gauged by acetylene-reducing performance (Halliday 1976), but in broad bean (*Vicia faba* L.) or white lupin, fixation continues unabated for several days after removal of the shoot, suggesting that carbohydrate reserves of the tap-root might be made available as a substrate for nitrogen fixation. Drought-induced defoliation can also cause root reserves to be utilized for nitrogen fixation (unpublished data).

It has recently proved possible to compare experimentally-obtained values for CO_2 efflux of nodules with theoretical estimates of the costs, in terms of respired CO_2 , of providing the ATP and reductant necessary for converting N_2 to NH_3 , and for assimilating this ammonia into the compounds known to be exported from the nodule (Atkins *et al.* 1978). A summary of the relevant calculations (Table 3) shows that, for the three grain legumes studied, estimates of CO_2 output based on the theoretical requirements for ATP by the nitrogenase system and ammonia assimilating reactions exceed by a substantial margin the experimentally-observed CO_2 output

of the nodules. The differences involved appear to be greater in one legume (cowpea) than in the other two (garden pea and white lupin). The discrepancy between observed and predicted CO_2 efflux would be partly resolved were the nodule to economise in its carbon usage by re-fixing some of its respired CO_2 , and to utilize this carbon in synthesis of the carbon skeletons of the compounds employed in transporting fixed N to the shoot. The demonstration of an active phosphoenol-pyruvate carboxylase system in nodules of *Lupinus angustifolius* L. by Christeller *et al.* (1977) suggests that nodules assimilate CO_2 , and do so at a sufficiently fast rate (up to 0.8 molecules of CO_2 incorporated per atom of N fixed) to effect a considerable saving in terms of imported carbon from the shoot. It remains to be seen whether this applies to legumes generally.

The most likely source of discrepancy between observed and predicted values for CO_2 output from N-fixing nodules relates to the uncertainty of the *in vivo* requirements of the nitrogenase for ATP. The estimates of Table 3 assume that 4 ATP molecules are needed per electron pair utilized by nitrogenase, a value regarded as an average estimate for the performance of isolated nitrogenase *in vitro* (Dilworth 1974). In living bacterial tissue nitrogenase might operate at much lower cost since the structural configuration of the enzyme system *in vivo* might not require the continuous stabilization involving ATP hydrolysis needed to maintain activity of the extracted enzyme (Atkins *et al.* 1978).

Table 3.

Theoretical estimates of and experimental observations on CO₂ production by nitrogen-fixing nodules of three grain legumes.

	<i>Vigna</i> <i>Lupinus</i> <i>Pisum</i>		
(A) Theoretical Estimates ⁽¹⁾			
	mol ATP . mol NH ₃ ⁻¹		
1. ATP requirements for synthesis of organic solutes of nitrogen from NH ₃ ⁽²⁾ —			
amino acids	0.40	0.84	0.48
glutamine-amino	0.20	0.24	0.30
glutamine-amido	0.05	0.06	0.08
asparagine-amino	0.20	1.34	1.26
asparagine-amido	0.15	1.01	0.95
ureide	2.10	—	0.30
Total cost for NH ₃ assimilation	3.10	3.49	3.37
2. CO ₂ output associated with nitrogen assimilation ⁽³⁾			
N ₂ reduction to NH ₃	1.75	1.75	1.75
NH ₃ incorporation into organic N solutes	0.52	0.58	0.56
Total N assimilation cost	2.27	2.33	2.31
3. CO ₂ output for growth and maintenance.			
C loss as CO ₂ in nodule formation ⁽⁴⁾	0.06	0.06	0.11
C loss as CO ₂ in nodule maintenance ⁽⁵⁾	0.05	0.06	0.01
Total nodule cost	0.11	0.12	0.12
4. Total CO ₂ output.			
Items 2 and 3	2.38	2.45	2.43
(B) Experimental Observations.			
	mol CO ₂ . mol NH ₃ ⁻¹		
Output of respired CO ₂ from nodule	1.27	2.02	1.73

(1) As detailed by Atkins *et al.* (1978).

(2) Synthetic pathways as described by Atkins *et al.* (1978), proportions of amino compounds produced as suggested from xylem sap analysis.

(3) Calculated as 6 ATP per CO₂, assuming that the P/2e⁻ ratio of oxidative phosphorylation is 3.

(4) Calculated using 0.2 g CO₂ produced .g dry wt⁻¹ synthesized (Penning de vries *et al.* 1974).

(5) Calculated using 30 mg glucose .g dry matter⁻¹ day⁻¹ (from Penning de Vries 1975).

(6) From studies of Minchin and Pate 1973 (*Pisum*); Herridge and Pate 1977 (*Vigna*); Pate and Herridge 1978 (*Lupinus*).

An important factor in the efficiency of functioning of nitrogenase relates to its activity in converting protons to hydrogen gas, a process which will compete for electrons with the reduction of nitrogen gas to ammonia (Dixon 1975). In nodules formed by certain legume-*Rhizobium* associations hydrogen evolution is high relative to ammonia formation; in others a hydrogenase system re-utilizes much of the hydrogen which is produced, the attendant conversion to water generating significant amounts of ATP (Dixon 1975; Schubert and Evans 1976). It follows that nodules possessing an active hydrogenase system will have a lower requirement for ATP than those not so endowed, and that their overall consumption of carbon in nitrogen fixation will

be correspondingly reduced. It appears that the strain of *Rhizobium*, not the host plant, is responsible for determining the level of hydrogenase in the nodules of a particular association (Schubert *et al.* 1977) but that ontogenetic and environmental factors may modify H₂ evolution from nodules during the growth cycle of a specific association (Bethlenfalvay and Phillips 1977). This suggests that nodules may not operate at a constant level of hydrogenase activity relative to nitrogen fixation. There is still considerable confusion surrounding the significance of hydrogen evolution and hydrogenase activity in nodule functioning, but it would be especially interesting to see whether the apparent differences in efficiencies of carbon usage by the N-fixing associations in Table 2 relate to effects of this nature.

The structure and functioning of fruits

Grain legumes display great variation in size, shape and structure of their fruits. In some (e.g. peanut (*Arachis hypogaea*) and bambarra ground nut (*Voandzeia subterranea* Thon.) the fruits develop underground and lack chlorophyll. In most, however, the fruits are green and are borne aerially, often in a well-illuminated situation in the canopy. They thus have the potential for photosynthesis and for attracting water and nutrients from the parent plant through their transpirational activity.

The legume fruit consists of a single carpel containing 1-10 or more seeds. Its vascular network comprises a ventral longitudinal strand, two dorsal longitudinal strands supplying the seeds, and a network of veins traversing the lateral walls of the pod and connecting above and below with the longitudinal strands. A gas cavity of variable size and shape forms within the carpel as the young fruits develop. During later growth this gas space is encroached upon by the developing seeds (Harvey *et al.* 1976; Flinn *et al.* 1977).

Anatomists recognise three basic layers in the pod wall of legumes—exocarp, mesocarp and endocarp (see Esau 1965; Fahn 1974; Fig. 2). The exocarp comprises a heavily-cutinized epidermis, poorly supplied with stomata, and, in some genera, an underlying hypodermis of lignified cells. The mesocarp consists of 5-40 layers of thin-walled, highly vacuolated cells, usually well supplied with plastids. The outer mesocarp contains the bulk of the chlorophyll and displays prominent air-spaces, especially adjacent to stomata (Fig. 2). The inner mesocarp shows few, if any, air spaces and contains the vasculature of the pod wall. The endocarp consists of one or more layers of sclerenchyma, an inner epidermis, and, in some genera, an intervening zone of parenchyma. Since cultivated legumes have been selected for non-shattering pods the fibrous component of the endocarp is usually much reduced in comparison with ancestral forms of the species (Zimmermann 1936). In certain grain legumes, for example the "mange tout" forms of garden pea, sclerenchyma may be completely absent, making the fruits soft and edible.

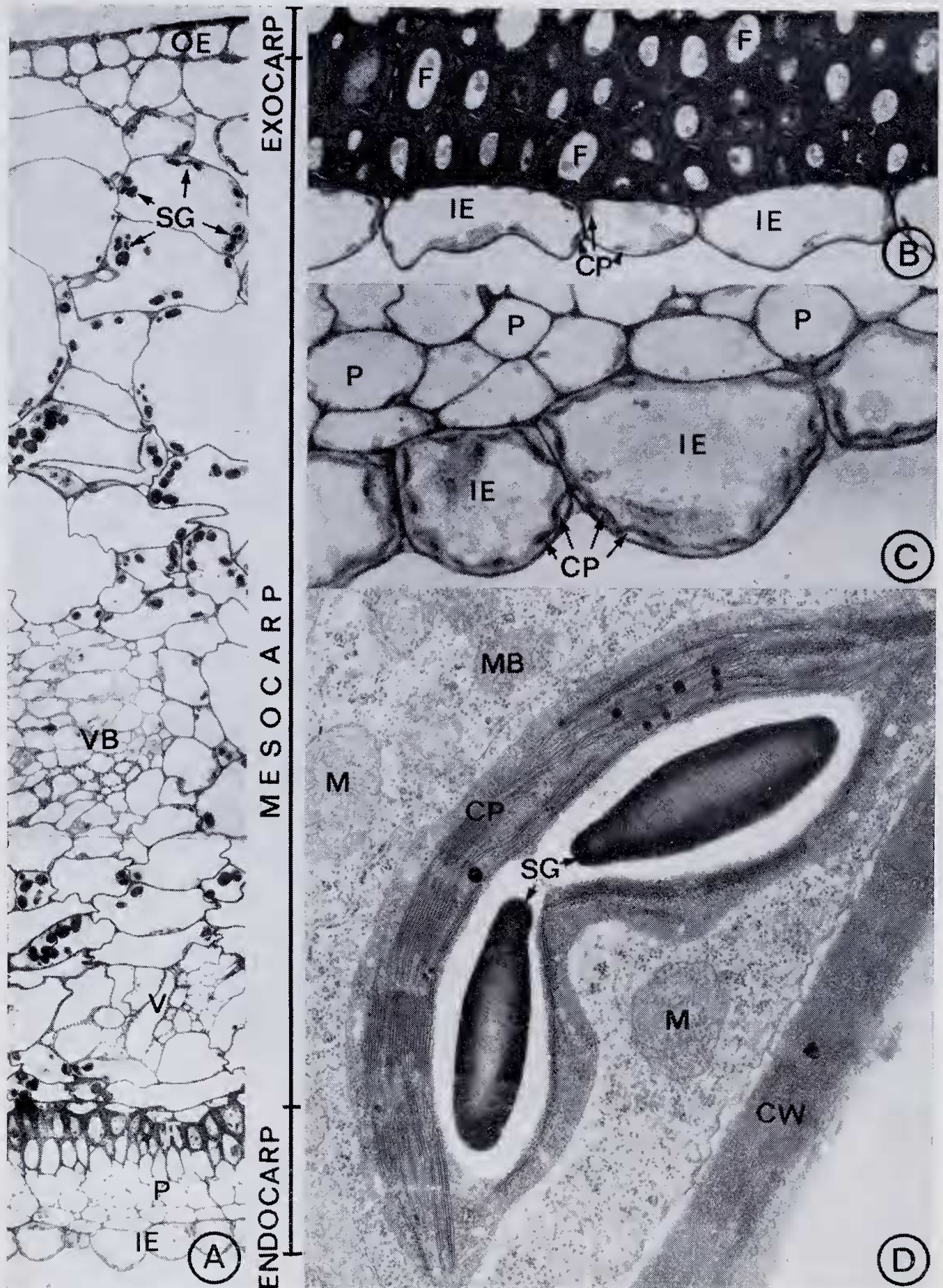


Figure 2.—Pod wall structure in grain legumes. (A) Transverse section of the pod of *Pisum sativum* L. showing wall layers (x195). (B) Endocarp of pod of *Lupinus albus* L. (x430). (C) Endocarp of pod of *Pisum sativum* (x850). (D) Electron micrograph of portion of cell of photosynthetic inner epidermis of the pod of *Pisum sativum* showing chloroplast (x24 000). OE—outer epidermis; SG—starch grain; VB—vascular bundle; V—inward projecting veinlet of vascular network; F—fibre layer; P—parenchyma of endocarp; IE—inner epidermis (photosynthetic); CP—chloroplasts of inner epidermis; M—mitochondria; MB—microbody; CW—cell wall. (Photographs kindly supplied by Dr. J. Kuo, Botany Department, University of Western Australia).

The pod walls of grain legumes are far from opaque, with up to half of the photosynthetically-active radiation received at the pod surface being transmitted to the inner epidermis (Atkins *et al.* 1977). In many genera (e.g. *Lupinus*, *Pisum*, *Vigna*, *Glycine*, *Cajanus*, *Cicer*) the cells of the inner epidermis are equipped with chloroplasts (Kuo and Pate unpublished; Fig. 2) and in some cases (e.g. *Pisum sativum* and *Lupinus albus*) the free-ending veinlets of the vasculature of the pod wall project inwards from the surface of the endocarp, an adaptation, one might suppose, for collection of assimilates produced by the photosynthetic endocarp. In *Lupinus albus* these veinlets consist solely of phloem (Kuo and Pate unpublished) a feature consistent with a retrieval function within the pod wall.

Several physiological studies on grain legume fruits have provided information directly relevant to pod structure. Rates of exchange of CO₂ and transpiration rates are low, in comparison with leaves, as suggested by the structural features of the outer epidermis of the pod (Flinn and Pate 1970; Crookston *et al.* 1974; Andrews and Svec 1975). Poor ventilation also results in high concentrations of CO₂ (0.05-2.5% by volume of the fruit's gas space) (Harvey *et al.* 1976; Atkins and Pate 1977), much of this CO₂ being attributed to respiration of the developing seeds. Since CO₂ levels in the gas space are lower in illuminated than in darkened fruits, photosynthetic recycling of carbon by the internal tissues of the fruit is suspected. In *Pisum sativum*, a species with chloroplasts in its inner epidermis, injection of ¹⁴CO₂ into the gas cavity of an illuminated fruit leads to labelling of the pod but not the seeds, but neither pod nor seeds become labelled if the ¹⁴CO₂ injected into a fruit in darkness (Flinn *et al.* 1977). Labelled assimilates formed from ¹⁴CO₂ injected into an illuminated fruit can be traced first to the vascular network of the pod and then to the seeds, confirming the pod's role in recycling respired carbon back to the seeds.

Up to 25% of the chlorophyll of the pod of garden pea is contained in the inner epidermis, and this single layer also contains significant fractions of the pod's CO₂-assimilating enzymes, ribulose diphosphate carboxylase and phosphoenolpyruvate carboxylase (Atkins *et al.* 1977). The last of these two enzymes may function particularly well in the CO₂-rich environment of the endocarp, and it is significant that the same enzyme occurs in high concentration in seeds of certain grain legumes (Hedley *et al.* 1975; Atkins and Flinn unpublished data).

Using data for compositional changes in pod and seed during growth, information on the day by day exchange of CO₂ and transpiration of the fruit, and analysis of the xylem and phloem fluids supplying the fruit with water and assimilates, it has proved possible to construct a quantitative model of a legume fruit's functioning based on its intake of carbon, nitrogen and water through xylem and phloem. The model for white lupin, as described by Pate *et al.* (1977) (see Fig. 3), indicates that 93% of the imported

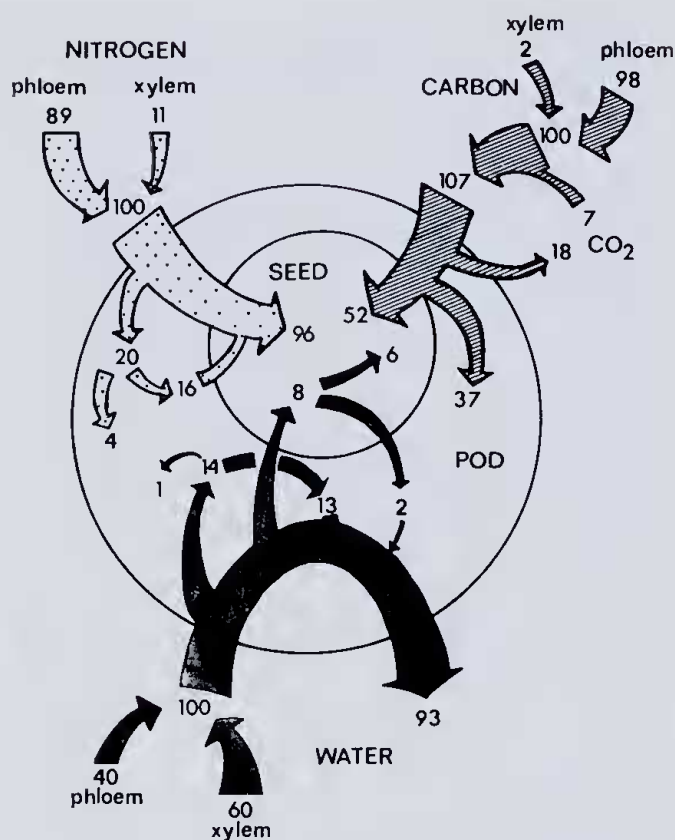


Figure 3.—Economy of carbon, nitrogen and water in a fruit of lupin (*Lupinus alba* L. cv Neutra). Deliveries through xylem and phloem are expressed relative to a net intake of 100 units of a specific commodity. Net photosynthesis, respiration loss, transpiration, water losses from tissues during fruit ripening and nitrogen mobilization from pod to seed are depicted. The weight ratio of imports over the life of the fruit is C:N:H₂O::12:1:600. (from Pate *et al.* 1977; Sharkey 1977).

carbon, 89% of the nitrogen and 40% of the water enters through phloem, the remainder through xylem. A fully-illuminated fruit effects net gains of carbon during the photo-periods of all but the last two weeks of its life (Sharkey 1977), but in no week of its life do the net daily gains of CO₂ from the outside atmosphere compensate for the night time loss of CO₂ from the fruit. Net respiratory losses increase dramatically as the fruit ages, due to increasing CO₂ output from the seeds and loss of chlorophyll and of photosynthetic performance by the pod.

Of every 100 units of carbon imported by a white lupin fruit over its life, 52 are eventually incorporated into seeds, 37 into non-mobilizable materials of the pod, and the remaining 11 lost as a net efflux of CO₂ to the outside atmosphere. By contrast 96% of the nitrogen entering the fruit ends up in the seeds, this element being mobilised to the seeds from temporary storage in the pod with 80% efficiency (Fig. 3).

The heavy dependence of legume fruits on phloem-borne assimilates and their low attraction of xylem-mobile solutes through transpiration, results in fruits, and especially seeds, having a final composition closely resembling that of phloem translocate (Pate *et al.* 1977; Hocking and

Pate 1977). In particular they exhibit low levels of elements only sparingly mobile in phloem (e.g. Ca and Mn), and under conditions of shortage of these elements seeds are likely to be the first to show deficiency symptoms. This is well displayed for manganese deficiency in the "marsh spot" disorder of peas (Reynolds 1955) and for the split-seed syndrome associated with deficiency of the same element in lupins (Perry and Gartrell 1976; Hocking *et al.* 1977). Similarly a reduction in transpiration of a fruit by enclosure can cause deficiency symptoms of calcium to occur in fruits, suggesting how critically important the transpirational attraction of xylem-mobile nutrients is in the normal development of the fruit (see Wiersum 1966). In underground fruits of legumes such as peanut the supply of Ca through the fruit stalk is supplemented by absorption of the element by the fruit surface from the rooting medium (Bledsoe *et al.* 1949), a property compensating for the meagre xylem intake of the fruit.

Our studies of CO₂ fluxes between the pea fruit and its external and internal atmospheres, have enabled its photosynthetic performance to be related to the overall carbon budget of the fruit. During the first half of a 36-day growth period (Fig. 4A; 0-18 days) much of the carbon imported through the fruit stalk is consumed in pod growth, and ¹⁴CO₂ feeding studies show that pod photosynthesis is to the benefit of the pod rather than the contained seeds. At this time the pod fixes more CO₂ from the external atmosphere than from the pod gas space, largely because the respiratory output of seeds at this stage is small relative to the photosynthetic potential of the pod. In the second half of fruit development (18-36 days; Fig. 4B), pods have ceased to accumulate dry matter so translocate from the parent plant is directed entirely to seeds. Seeds also benefit from pod photosynthesis, especially the fixation of respired CO₂ from the gas cavity of the fruit.

As a result of pod photosynthesis a fully illuminated fruit of *Pisum sativum* requires over its life 16-19% less carbon from the parent plant than would a fruit laying down the same amount of dry matter in total darkness (Flinn *et al.* 1977). It follows that agronomic practices should be designed to maximise the efficiency of light interception by fruits in the plant canopy.

From the viewpoint of seed production as a nutritional source for animals the most significant measure of fruit performance is how efficiently assimilates imported from the parent plant are ultimately converted into digestible food reserves of seeds. To make such estimates we require knowledge of the weight of sugar and nitrogenous solutes imported by the fruit during its life, the final dry weight of the seeds, and the proportion by weight therein of "useful" food reserves such as starch, oil and protein.

A comparison between two species, white lupin (Pate *et al.* 1977) and garden pea (Flinn *et al.* 1977), shows how grain legumes may vary in these respects. Both of these species bleed from their phloem and xylem (Pate *et al.* 1974), so the

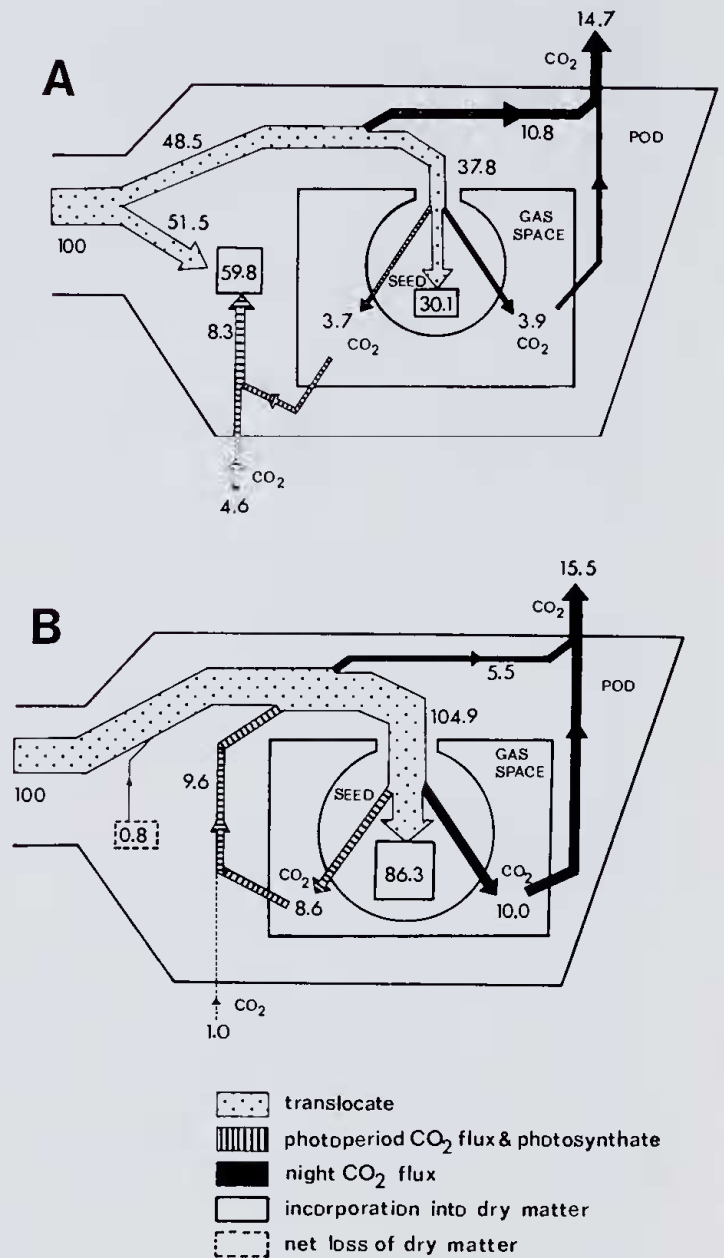


Figure 4.—Economy of carbon in developing fruits of *Pisum sativum* L. (cv Greenfeast). Carbon fluxes are shown in relation to intake of translocate and exchanges of CO₂ between seeds and pod of the internal and external atmospheres of the fruit. Day-time respiration of the fruit is not shown. (A) 0-18 days after anthesis, a period when 351 mg C were imported through the fruit stalk; (B) 18-36 days after anthesis, a period when 797 mg C were imported through the fruit stalk. The flux values in (A) and (B) are expressed relative to an intake of 100 units of carbon through the fruit stalk. (Data from Flinn *et al.* 1977).

identity of the translocated solutes received by the fruit can be accurately assessed. This information, matched against the economy of carbon, nitrogen and water of the fruit, allows one to compute the precise weights of amides, amino acids and sugars imported by the fruit during its life. As shown in the data of Table 4 the pea fruit turns out to be the more efficient converter of assimilates into seed dry matter (69% in peas, 50% in lupin), or "useful" food

substances (45% in peas versus 31% in lupin), but the lupin fruit emerges as the superior species if efficiency is measured in terms of production of seed protein.

Table 4.

Efficiency of conversion of translocate into seed products by fruits of two species of grain legume

	<i>Pisum sativum</i> L. (cv Greenfeast)	<i>Lupinus albus</i> L. (cv Neutra)
(A) Solutes translocated to fruits (mg . plant⁻¹)		
sucrose	2 404	1 756
amides + amino acids	393	334
(B) Materials laid down in fruits (mg . plant⁻¹)		
total dry matter	1 930	1 070
protein	387	412
oil	19	132
perchloric acid-soluble carbohydrate	857	110
(C) Conversion efficiency by weight of imported solutes to fruit components (%).		
to dry matter	69	50
to protein	14	19
to protein + oil + digestible carbohydrate	45	31

It remains to be seen how the values obtained by the two fruits described above compare with grain legumes as a whole, and whether better conversion efficiencies might be achieved in fruits by careful selection or breeding.

The carbon economy of whole legumes

Before judgment can be passed on the relative efficiencies with which grain legumes operate as crop plants, assessments must be made of their total performance, especially in the context of utilization of photosynthate, for nitrogen fixation and, ultimately, for seed production. With this in mind we have attempted to construct for several grain legumes budgets for carbon utilization during growth of the whole plant and its parts, utilizing information on carbon gains or losses as dry matter of plant parts, knowledge of the compounds involved in root-shoot interchange via xylem and phloem, and assessments of respiratory and photosynthetic exchanges of CO₂ by plant organs over specific intervals of the growth cycle. The budgeting takes as its centrepoint the partitioning of the net photosynthate produced by photosynthetic surfaces of the plant during the day, this quantity being determined indirectly as:—

$$\begin{matrix} \text{Net photosynthate} & & \text{Total C gain as dry} & & \text{Respiratory loss} & & \text{Respiratory} \\ \text{(net C gain by} & = & \text{matter by plant day} & + & \text{of C from} & + & \text{loss of C by} \\ \text{shoot in day)} & & \text{and night} & & \text{shoot at night} & & \text{nodulated root} \\ & & & & & & \text{(day + night)} \end{matrix}$$

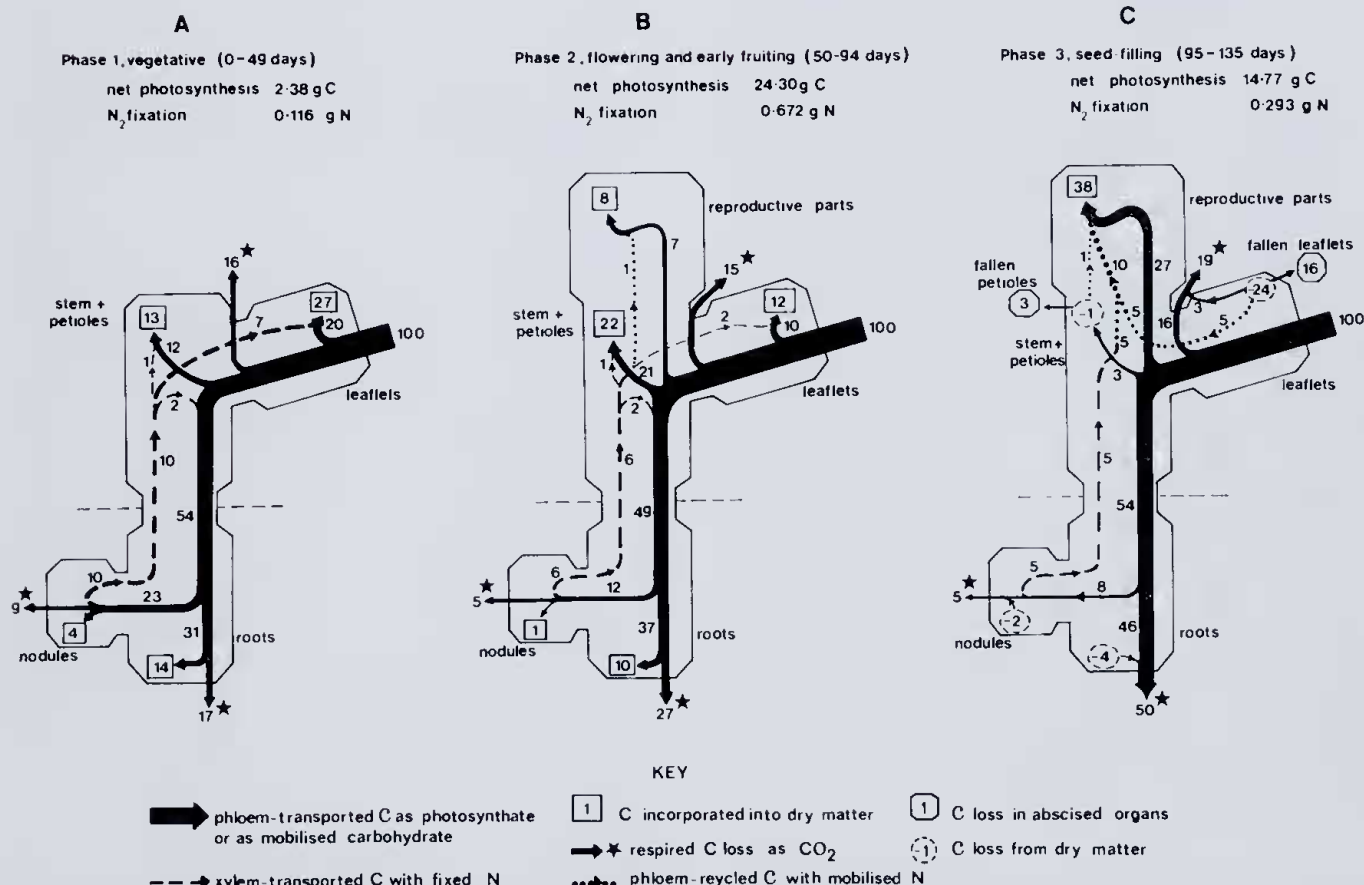


Figure 5.—The economy of carbon in nodulated plants of *Lupinus albus* L. (cv Neutra), relying solely on nodules for their nitrogen supply. The situation in three phases of the growth cycle is described. In each case items of the carbon budget are expressed relative to a net intake of 100 units of carbon by photosynthetic surfaces of the shoot. The amounts of carbon and nitrogen assimilated during each phase are indicated. (Data from Pate and Herridge 1978).

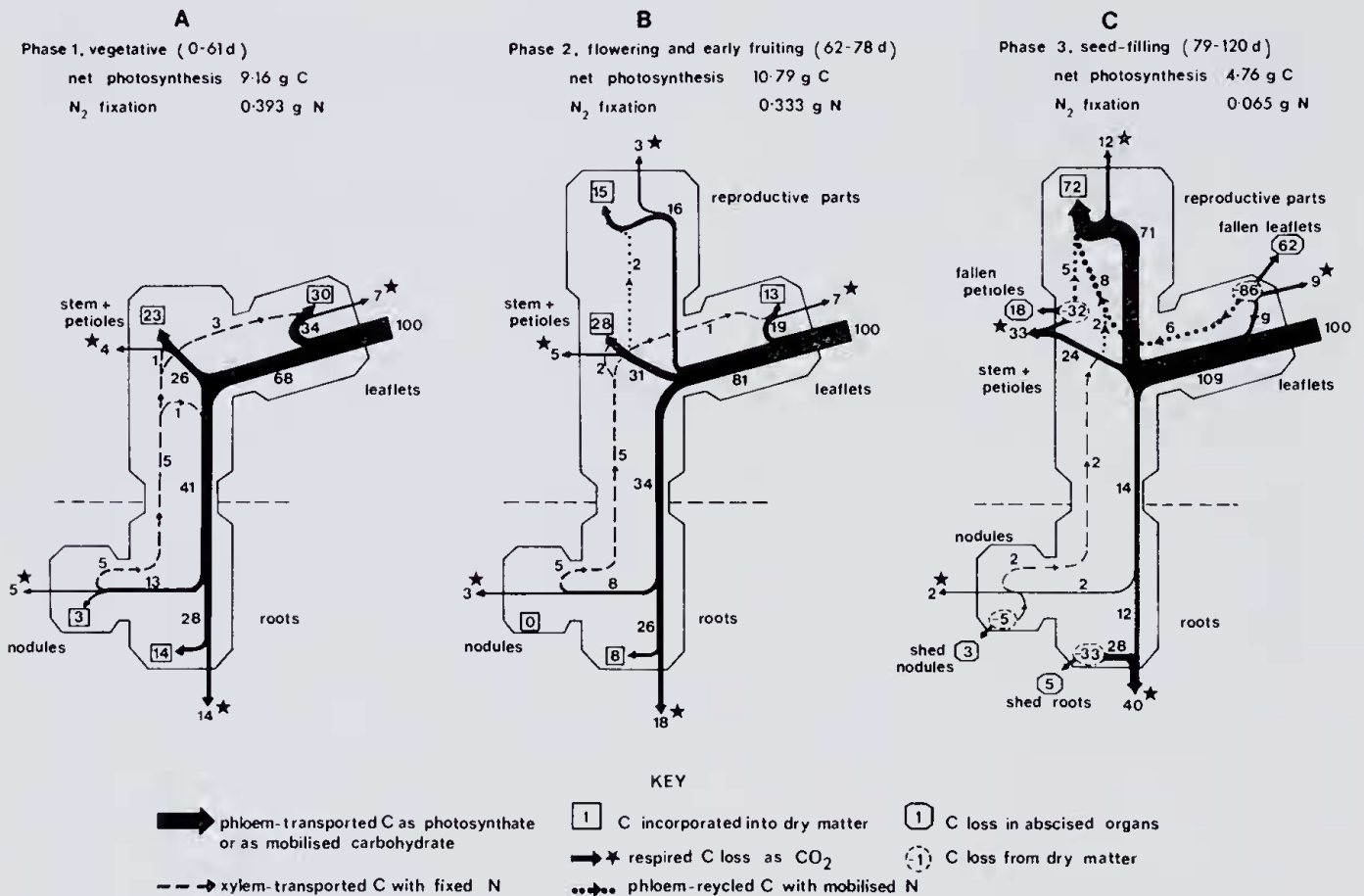


Figure 6.—The economy of carbon in nodulated plants of *Vigna unguiculata* (L.) Walp. (cv Caloona) relying solely on nodules for their nitrogen supply. The situation in three phases of the growth cycle is described. In each case items of the carbon budget are expressed relative to a net intake of 100 units of carbon by photosynthetic surfaces of the shoot. The amounts of carbon and nitrogen assimilated during each phase are indicated. (Data from Herridge and Pate 1977).

By measuring respiratory losses and dry weight changes in nodules and roots separately from those of shoots, the significance of the plant's nodules as consumers of net photosynthate can be assessed. Also, as described in an earlier section on nodule functioning, the C:N ratio of xylem exudate can be related to nitrogen increments of the shoot to estimate how much of the carbon donated to the root, as photosynthate, returns back to the shoot attached to products of nitrogen fixation.

Information is available at present for only two legumes, white lupin, a tap-rooted species, grown as a winter annual in Western Australia, and cowpea, a slender-rooted tropical legume and a summer legume as far as culture in Western Australia is concerned. The cultivars of both species used for the experiments were characterized by low harvest indices for seed production, and hence may be regarded as typical of the many legumes needing selection for improved yield of grain.

The carbon economy of these species is summarized in Figures 5 and 6, the comparisons relating to each of three stages of growth, namely vegetative development prior to flowering, flowering and early fruiting, and seed filling. Each budget expresses items of expenditure of

carbon relative to a net intake of 100 units of carbon as net photosynthate by foliar surfaces of the plant. Attached to each budget is information on the amounts of nitrogen fixed and net photosynthate generated, so that the relative importance of each phase of growth is depicted relative to the total assimilatory output of the plant during its life. Comments relating to each phase of development are as follows:—

Vegetative growth.—Although the vegetative phase of lupin lasted longer and encompassed a much greater proportion of the plant's growth and assimilation than in cowpea, the species show very similar patterns of partitioning of net photosynthate. The largest single item is carbon translocated to roots (54% of net photosynthate in lupin, 41% in cowpea), most of this being respired, some contributing to growth of root and nodule, and a relatively small amount returning to the shoot associated with products of nitrogen fixation.

Flowering and early fruiting.—Both species show greatest photosynthetic and nitrogen-fixing capacities at this time. Lupin continues to acquire almost half (49%) of the plant's net photosynthate, versus one-third (34%) in cowpea. The high consumption of carbon by roots of lupin relates particularly to the establishment

and maintenance of an extensive tap-root. Whilst most desirable for growth in the dry, nutrient-poor sands of south-west Australia, this feature clearly carries penalties in terms of decreased diversion of photosynthate to grain.

The proportions of photosynthate consumed by nodules during flowering and early fruiting is less than in vegetative growth, reflecting the ever increasing sink capacity of shoots for photosynthate. Four times more carbon is bound into shoots than into nodulated roots in lupin, seven times more in the case of cowpea, so the shoot: root weight ratio widens progressively after flowering.

Seed filling.—Most marked differences occur between the species at this stage. The roots share of photosynthate remains high (54%) in lupin, but is much less (14%) in cowpea. As might be expected, returns of fixed N from nodules are lower during this phase in cowpea than in lupin, and, due to a lower expenditure of carbon on roots, cowpea is able to divert relatively more photosynthate to fruits than in lupin. However, when measured in terms of efficiency of mobilization of nitrogen to seeds, lupin proves superior, 61% of its fixed nitrogen ending up in seeds compared with only 43% in cowpea. This is because the high protein seeds of lupin incorporate relatively more nitrogen than do the low protein seeds of cowpea (see Tables 1 and 5).

Since the value of legumes stems mainly from the protein which they synthesize, the efficiency with which photosynthate is used for this purpose is a very relevant measure of their functioning as crop plants. The cultivars of cowpea and lupin examined in our investigations are used in agriculture as green forage crops as well as for grain production, so conversion efficiencies are appropriate if based on photosynthate consumption in relation to protein synthesized in above-ground vegetative parts by the time of flowering, or on the basis of protein harvested in seeds at the end of the growth cycle. The relevant calculations are shown in Table 5, where it can be seen that 17-25 g net photosynthate (as carbohydrate) are required to make each gram of protein accumulated in above ground parts by the time of flowering, 31-33 g carbohydrate for each gram of protein harvested as seed. Thus there is a conversion by weight of only 4-5.8% of the net photosynthate produced in vegetative growth into forage protein, an efficiency of only 3% for the comparable conversion to seed protein. It would be especially interesting to see how the values for the relatively unimproved cultivars studied here compare with the situation in cultivars and species of grain legumes selected for high yield of seed. Such comparisons are in progress.

It is now appropriate to return to the original question of how grain legumes compare with other seed-yielding crops in productivity and overall efficiency of performance. There simply is not yet enough data available for such comparisons to be made, since the economy of carbon in non-legumes has yet to be studied on strictly comparable grounds to our own studies of legumes, and it would indeed be most unwise to attempt to generalize about legume performance

Table 5.

Costs in terms of net photosynthate of protein production by two grain legumes ⁽¹⁾

	<i>Vigna unguiculata</i> (L) Walp. (cv Caloona)	<i>Lupinus albus</i> L. (cv Neutra)
(A) Synthesis of protein in above-ground vegetative parts ⁽²⁾ .		
Production of net photosynthate (g carbohydrate . plant ⁻¹)	50.1	66.7
Protein accumulated in shoot (g . plant ⁻¹)	2.91	2.70
Net photosynthate consumed per unit of protein synthesized (g carbohydrate . g protein ⁻¹)	17.2	24.7
(B) Synthesis of seed protein ⁽³⁾ .		
Production of net photosynthate (g carbohydrate . plant ⁻¹)	61.8	103.6
Protein accumulated in seed (g . plant ⁻¹)	1.90	3.34
Net photosynthate consumed per unit of seed protein synthesized (g carbohydrate . g protein ⁻¹)	32.5	31.0

(1) Data for cowpea from Herridge and Pate (1977) and for white lupin from Pate and Herridge (1977).

(2) Measured over the period from germination to the time of maximum nitrogen content in above-ground vegetative parts (i.e. at 79 days after germination in *Vigna*, and at 95 days in *Lupinus*).

(3) Production of net photosynthate calculated for complete growth cycle, seed protein measured as amount present per plant at full maturity (i.e. at 120 days in cowpea, and at 135 days in white lupin).

on the basis of investigations of only two species. It would seem appropriate in further studies on legumes to examine the mechanisms underlying the substantial respiratory losses which occur from roots and the processes whereby fruits and nitrogen-fixing nodules compete for a dwindling supply of assimilates at the end of the growth cycle. Our data suggest that nodules, and their fixation activities, are not particularly large consumers of photosynthate, so that any inadequacies which legumes may show in comparison with non-legumes are likely to relate to the overall performance of the host plant rather than to the symbiotic fixation of nitrogen.

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